PATENT

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Nicky McKinnon
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Gary Ruvkun et al.

Art Unit: 1633

Examiner: Sumesh Kaushal, Ph.D.

Serial No.: 09/205,658

Filed: December 3, 1998

Title: THERAPEUTIC AND DIAGNOSTIC TOOLS FOR IMPAIRED GLUCOSE TOLERANCE CONDITIONS

BOX AF
Commissioner For Patents
Washington, D.C. 20231

DECLARATION UNDER 37 CFR §1.132 OF GARY RUVKUN, PH.D.

I declare:

1. I am an inventor on the above-captioned patent application.

2. I have read the Office Action mailed January 10, 2000.

3. It is my opinion that a person of ordinary skill in the field of *C. elegans* genetics could have broadly practiced the invention as claimed by using the teachings in the patent application in combination with the knowledge and techniques known in the field at the time the application was filed.

4. The regulatory regions of *daf-18* can be readily obtained using techniques known to a person skilled in molecular biology. For example, at the time the application was filed, genomic library screening methodologies, PCR techniques, and genomic sequence database searches were commonly used by molecular biologists to obtain 5' flanking regions of a desired gene. Once the 5' flanking region is obtained, deletion mutants can readily be analyzed to identify the important regulatory regions.

5. These standard techniques have, in fact, been used to clone the *daf-18* promoter. Using standard PCR techniques, a 6.9 kb DNA fragment was isolated from *C. elegans* N2 genomic DNA. This DNA fragment contains the *daf-18* gene as well as approximately 1.0 kb of 5' flanking sequence and approximately 1.1 kb of 3' flanking sequence. These flanking sequences contain regulatory sequences that are sufficient to direct expression of *daf-18* and to rescue a *C. elegans daf-18* mutant.

6. My laboratory has demonstrated that human PTEN and *C. elegans daf-18* function similarly by showing that PTEN rescues *C. elegans daf-18* mutants. The experiment was performed as follows. The human PTEN cDNA was placed under the control of approximately 1.0 kb of *daf-18* 5' flanking sequence and approximately 2.4 kb of *daf-18* 3' flanking sequence. Transgenic *C. elegans* (*daf-2*;*daf-18* double mutants) containing this minigene DNA were generated using standard methods known at the time the present application was filed. The F2 progeny of the transgenic *C. elegans* were then evaluated for restoration of the Daf phenotype (dauer formation at 25°C, but not at lower

temperatures, for example, 20°C), which would indicate that rescue of the *daf-18* mutation was successful. The results of these studies were that human PTEN fully rescued the phenotype of the *daf-2*;*daf-18* double mutants.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

7/10/00
Date

Gary Ruvkun, Ph.D.

\\Ntserver\documents\00786\351xxx\00786.351004 Declaration of Dr. Ruvkun for O.A. mailed 1.10.00.wpd